REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.116, are respectfully requested.

By the foregoing amendment, the specification has been amended on page 1 to modify the continuing data information and on page 2 to modify recitation of the appropriate sequence identifiers as requested by the examiner to be separately recited after each peptide. Currently pending claims 15 and 16 have also been amended. In particular, claim 15 has been amended to correct the inadvertent typographical error which was introduced when applicants attempted to recite 65°C (not 65%). Claim 16 has been amended to delete the term "stringent". Further, claims 15 and 16 have also been amended to recite "nucleotides 1 to 510 of SEQ ID NO: 7" (see, for example, page 9, lines 29-35 for support) and to recite washing conditions (see at least page 8, lines 28-32 for support). No new matter has been added.

Turning now to the Official Action, the Examiner has maintained the objection to the specification for not complying with the Sequence Rules. Applicants respectfully traverse this rejection as the sequences on page had been properly identified by their sequence identifier number. However, to expedite prosecution and not to acquiesce to the Examiner's objection, page 2 of the specification has been amended to recite the appropriate sequence identifier number of each of formulas II-VII as opposed to simply reciting "(SEQ ID NOS: 1-6)" prior to each formula be listed. In view of the above, the Examiner is respectfully requested to withdraw this objection to the specification.

The Examiner has objected to the Amendment and Reply filed on December 17, 2003 under 35 U.S.C. § 132 for allegedly introducing new matter into the specification. As described in the December 17, 2003 Amendment and Reply, M.P.E.P. § 1893.03(c), specifically states that the "it is not necessary for the applicant to amend the first sentence of the specification to reference the international application number . . . ." The same section of the M.P.E.P. also states that the filing date of the national stage application "is the filing date of that international application." Therefore, the Examiner's objection to the modification to the specification in the December 17, 2003 Amendment and Reply which corrected the filing date of application Serial No. 07/499,276 is incorrect. However, to expedite prosecution in the present application and not to acquiesce to the Examiner's objection, the continuing date has been amended to reference the international application number. In view of the above, withdrawal of this objection is respectfully requested.

Claims 15-16 have been rejected under 35 U.S.C. § 112, first paragraph, as supposedly containing subject matter which was not described in the specification in a such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time the application was filed. This rejection is respectfully traversed.

First, the Examiner has rejected claims 15-16 for failing to comply with the written description requirement since such claims have qualified the hybrization technique with the word "stringent." The Examiner is incorrect with regard to claim

15 as this claim has been amended to delete reference to the term "stringent." In connection with claim 16, applicants' have deleted recitation of such term.[1]

Second, the Examiner has rejected claim 15 for apparently not reciting the specific washing conditions set forth on page 8, lines 28-32. To expedite prosecution and not to acquiesce to the Examiner's rejection, claims 15 and 16 have been amended to recite the noted washing steps

In light of the above, the Examiner is respectfully requested to withdraw the written description rejection.

Finally, claims 15-16 remain rejected under 35 U.S.C. § 102(b) as purportedly being anticipated by Rosinski-Chupin et al. (PNAS, 85:8553-57 (Nov. 1988)). This rejection is respectfully traversed.

As discussed above, applicants believe that the Examiner's postion regarding the filing date of application Serial No. 07/499,276 is incorrect. Moreover, despite the fact a translated copy of FR 8813353 has been previously submitted to the United States Patent and Trademark Office in application Serial No. 07/499,276, the Examiner has indicated that a translated copy of FR 8813353 is not of record. For the Examiner's convenience, attached hereto is another copy of the translation of FR 8813353.

Further, Examiner Canella has stated:

> The essential disagreement appears that the instant SEQ ID NO:7 is not present in PCT/FR89/00523 as evidenced by the attachment provided in the prior Office [A]ction which indicates that the instant SEQ ID NO"7 differs from the sequence of the PCT/FR89/00523 by four nucleotides.

Official Action mailed March 2, 2004, at 3.

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[1] This amendment is not intended to be a "narrowing" amendment.

As shown in the sequence alignment provided by the Examiner, SEQ ID No. 7 differs from the sequence disclosed in international application PCT/FR 89/00523 by four G/C substitutions in positions 534, 558, 568 and 608 (see the enclosed page 11 of PCT/FR 89/00523 with underlined nucleotides). These nucleotides localize in the 3' end of SMR1 cDNA but do not pertain to the coding sequence.

The specification teaches that SMR1 ORF consists of 510 nucleotides (page 9, lines 30-31). It can be directly inferred from the sequence shown page 9 that this ORF corresponds to nucleotides 1 to 510 of this sequence, since the last codon "AAA" ends in position 510. This SMR1 ORF, nucleotides 1 to 510, is thus defined identically in the instant application and in PCT/FR 89/00 523. Accordingly, the ORF sequence of SMR1 (or nucleotides 1 to 510 of EQ ID NO: 7) as set forth in currently pending claims 15 and 16 is to benefit from the priority date of PCT/FR 89/00 523 and thus cannot be anticipated by Rosinski-Chupin et al.

Thus, withdrawal of the rejection under 35 U.S.C. § 102(b) is respectfully requested.

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this Amendment and Reply, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: July 2, 2004

By: _____
Susan M. Dadio
Registration No. 40,373

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

détail cet élément d'ADNc inséré.

La séquence de l'ADNc codant pour SMR1 et la séquence déduite de la protéine sont représentées ci-après

```
AAA CTG ACT GAC CAG AGA CCT TCT CAC CAG CAC ATT TCC CCG CTC AGA AGT TTC      54

                          1
                          Met Lys Ser Leu Tyr Leu Ile Phe Gly Leu Trp Ile
TCC AAG GGG CTA CCA AAG ATG AAG TCA CTG TAT TTG ATC TTT GGC CTG TGG ATC     108

                                20                                  30
Leu Leu Ala Cys Phe Gln Ser Gly Glu Gly Val Arg Gly Pro Arg Arg Gln His
CTT CTA GCA TGC TTC CAG TCA GGT GAG GGT GTC AGA GGC CCA AGA AGA CAA CAT     162

                                    40
Asn Pro Arg Arg Gln Gln Asp Pro Ser Thr Leu Pro His Tyr Leu Gly Leu Gln
AAT CCT AGA AGA CAA CAA GAT CCT TCA ACT CTT CCT CAT TAT CTT GGT CTT CAG     216

     50                                     60
Pro Asp Pro Asn Gly Gly Gln Ile Gly Val Thr Ile Thr Ile Pro Leu Asn Leu
CCT GAT CCC AAT GGT GGA CAA ATA GGA GTA ACA ATC ACT ATA CCC TTA AAT CTT     270

               70                                  80
Gln Pro Pro Arg Val Leu Val Asn Leu Pro Gly Phe Ile Thr Gly Pro Pro Leu
CAA CCA CCT CGT GTT CTT GTT AAT CTT CCC GGT TTT ATC ACT GGA CCA CCA TTG     324

                    90                                 100
Val Val Gln Gly Thr Thr Glu Tyr Gln Tyr Gln Trp Gln Leu Thr Ala Pro Asp
GTT GTA CAA GGT ACC ACT GAA TAT CAA TAT CAG TGG CAG CTA ACT GCT CCA GAC     378

                         110                                 120
Pro Thr Pro Leu Ser Asn Pro Pro Thr Gln Leu His Ser Thr Glu Gln Ala Asn
CCT ACA CCT CTA AGC AAT CCT CCT ACT CAA CTT CAT TCC ACA GAA CAA GCA AAT     432

                              130
Thr Lys Thr Asp Ala Lys Ile Ser Asn Thr Thr Ala Thr Thr Gln Asn Ser Thr
ACA AAA ACA GAT GCC AAA ATC TCC AAC ACT ACT GCG ACT ACC CAA AAT TCC ACT     486

    140
Asp Ile Phe Glu Gly Gly Gly Lys
GAT ATT TTT GAA GGT GGT GGC AAA TAATAAATTCCTTTTCGGCAGTTAGAATAGCATAAATCAA    549

AACACTGTGTAGTTTTCGGCGAAATAATCTTTAAAGGCTTGAGAAACAACCTTTACCCGCATTATAGAAAA    620

TGACAATAAAGAGCTAAGCAGCATTACACAGCAAAAAA                                    658
```

L'ADNc inséré a une longueur de 652 nucléotides, si l'on exclut le fragment poly(A). La séquence comporte un cadre ouvert de lecture de 510 nucléotides. Le seul ATG qui peut servir de codon d'initiation est situé 73 nucléotides en aval de l'extrémité 5' du clone d'ADNc.

IN THE UNITED STATES PATENT OFFICE

I, Jean-Louis Joseph Norbert METZGER, Ph.D., M.Sc., E.N.S.C.S., translator to Randall Woolcott Services plc of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

1. That I am a resident of the United Kingdom of Great Britain and Northern Ireland.

2. That I am well acquainted with the French and English languages.

3. That the attached is a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in France on 11 October 1988 under the number 88/13,353 and the official certificate attached hereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the patent application in the United States of America or any patent issuing thereon.

The 8th day of July 1992

FRENCH REPUBLIC

NATIONAL INSTITUTE OF INDUSTRIAL PROPERTY

P A T E N T S

UTILITY CERTIFICATES - CERTIFICATES OF ADDITION

CERTIFIED TRUE COPY

The Director of the National Institute of Industrial Property certifies that the industrial property titleright, corresponding to the attached application, was granted on 6 March 1992

Drawn up in Paris, -1 JUL. 1992

On behalf of the Director of the
National Institute of Industrial
Property
The Divisional Head
(signature)
Yves CAMPENON

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National public establishment created by law No. 51-444 of 19 April 1951
BA 154/210990

N I I P N A T I O N A L I N S T I T U T E O F I N D U S T R I A L P R O P E R T Y

Cerfa No. 55-1222

DUPLICATE OF THE APPLICATION

APPLICATION FOR (see ticked box)

- a [X] PATENT
- b [ ] UTILITY CERTIFICATE
- c [ ] CERTIFICATE OF ADDITION
- d [ ] DIVISIONAL APPLICATION
- e [ ] CONVERSION OF A EUROPEAN PATENT APPLICATION

for c, d, and e state exactly the nature, number and date of the initial or principal application

2 COMPULSORY OPTIONS at the time of filing (except for utility certificate)

THE APPLICANT REQUESTS THE DIFFERED FORMULATION OF THE DOCUMENTATION REPORT [ ] YES [X] NO

IF THE OPTION SELECTED IS NO AND IF THE APPLICANT IS A PHYSICAL PERSON HE REQUESTS THE GRADUATED PAYMENT OF THE TAX ON THE DOCUMENTATION REPORT [ ] YES [ ] NO

NATURE	NUMBER	DATE OF INITIAL OR PRINCIPAL APPLICATION

DATE OF SUBMISSION OF THE DOCUMENTS: 11.OCT.1988

NATIONAL REGISTRATON No.: 88/13,353

DATE OF FILING: 11 OCT. 1988

3 NAME AND ADDRESS OF THE APPLICANT OR THE REPRESENTATIVE TO WHOM ALL THE CORRESPONDENCE SHOULD BE ADDRESSED

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4 DATE OF THE GENERAL POWER OF ATTORNEY

5 REFERENCE OF THE CORRESPONDENT: 88-F218

6 TELEPHONE No. OF THE CORRESPONDENT: 48.74.92.22

7 TITLE OF THE INVENTION

Peptides and polypeptides derived from the submaxillary gland of the rat, corresponding polyclonal and monoclonal antibodies, corresponding hybridomas and uses of these products for diagnosis, for detection or therapeutic purposes.

8 APPLICANT: SURNAME AND CHRISTIAN NAMES (UNDERLINE THE SURNAME) OR NAME AND LEGAL CONSTITUTION

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TAXES PAID

- [X] ON FILING
- [X] ON DOCUMENTATION REPORT
- [ ] ON CLAIM TO PRIORITY
- [X] ON CLAIM (from the 11th onwards)

11 INVENTOR(S)
THE APPLICANT IS THE SOLE INVENTOR* [ ] YES
If the answer is no see explanatory note [X] NO

12 IF THE APPLICANT IS A PHYSICAL PERSON NOT SUBJECT TO REVENUE COLLECTION, HE REQUESTS OR HAS REQUESTED REDUCTION OF THE TAXES [ ] YES [ ] NO

13 PRIORITY DECLARATION COUNTRY OF ORIGIN	FILING DATE	NUMBER	

14 [ ] DIVISIONS [ ] ADDITIONS

PREVIOUS TO THE PRESENT APPLICATION No. No. No. No.

15 SIGNATURE OF THE APPLICANT OR HIS REPRESENTATIVE NAME AND POSITION OF SIGNATORY (illegible signature) CABINET LAVOIX	SIGNATURE OF THE RECEIVING OFFICIAL	SIGNATURE OF THE APPLICANT OF THE APPLICATION AT THE N.I.I.P (illegible signature)

* Tick the relevant box

Registration number of the application

88/13,353

INTERNATIONAL PATENT CLASSIFICATION

Int. Cl.: 5

Ignore these figures ↓

C07K 5/10 , 7/06 , 13/00 ,
A61K 37/02 ,
G01N 33/577 ,
C12P 21/08

DESIGNATION OF THE INVENTOR

(if the applicant is not the inventor or the sole inventor)

National Registration No.

88/13,353

Title of the invention:

Peptides and polypeptides derived from the submaxillary gland of the rat, corresponding polyconal and monoclonal antibodies, corresponding hybridomas and uses of these products for diagnosis, for detection or therapeutic purposes.

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CABINET LAVOIX
Date and 13.12.1988 signature(s) of the applicant(s) or of the representatives
(illegible signature)

Peptides and polypeptides derived from the submaxillary gland of the rat, corresponding polyclonal and monoclonal antibodies, corresponding hybridomas and uses of these products for diagnosis, for detection or therapeutic purposes.

The present invention relates to novel peptides which are maturation products of polypeptides secreted by the submaxillary gland of the rat and analogs of these peptides.

Many polypeptides having defined biological properties are synthesized in large quantities in the submaxillary gland (SMG) of rodents, and in particular in the SMG of the mouse. These proteins, comprising the nerve growth factor (NGF), the epidermal growth factor (EGF) and renin, have a certain number of properties in common. They are all synthesized in the same type of cell, namely the cells of the convoluted tubules GCT (granular convoluted tubular), in response to various hormonal stimuli, in particular to androgens. Furthermore, it is possible to observe the presence of these secretory proteins in the saliva of the mouse, and they are synthesized in the form of precursors which become active after maturation processes which may involve proteases of the kallikrein type. Some of these proteases of the kallikrein type are also synthesized in the SMG under the control of androgens.

Attempts at the characterization of the genes controlled by the androgens in the SMG of the rat have led the inventors to analyze the electrophoretic profile of the in vitro translation products of the mRNA of this tissue.

A specific mRNA of the submaxillary gland of the male rat has been isolated. This mRNA corresponds to a polypeptide which has been designated SMR_1. This polypeptide gives maturation products with physiological activity. The present invention relates mainly to these peptides and to other peptides having similar properties.

Thus the subject of the present invention is peptides of formula:

X-His-Asn-Pro-Y I

in which:

X represents a glutamine (Gln) or pyroglutamic acid (pyroGlu) residue,

Y represents an OH group or a residue of a basic amino acid.

The basic amino acids may be lysine or arginine.

More specifically, the subject of the present invention is peptides of formula:

Gln-His-Asn-Pro II

pyro-Glu-His-Asn-Pro III

Gln-His-Asn-Pro-Arg IV

pyro-Glu-His-Asn-Pro-Arg V

Gln-His-Asn-Fro-Lys VI

pyro-Glu-His-Asn-Pro-Lys VII

Another subject of the present invention is the polypeptide SMR_1 which gives the maturation products of formulae II, III, IV and V. This polypeptide corresponds to the formula:

1

Met Lys Ser Leu Tyr Leu Ile Phe Gly Leu Trp Ile Leu

20

Leu Ala Cys Phe Gln Ser Gly Glu Gly Val Arg Gly Pro

30

Arg Arg Gln His Asn Pro Arg Arg Gln Gln Asp Pro Ser

40 50

Thr Leu Pro His Tyr Leu Gly Leu Gln Pro Asp Pro Asn

60

Gly Gly Gln Ile Gly Val Thr Ile Thr Ile Pro Leu Asn

70

Leu Gln Pro Pro Arg Val Leu Val Asn Leu Pro Gly Phe

80 9 90

Ile Thr Gly Pro Pro Leu Val Val Gln Gly Thr Thr Glu

100

Tyr Gln Tyr Gln Trp Gln Leu Thr Ala Pro Asp Pro Thr

110

Pro Leo Ser Asn Pro Pro Thr Gln Leu His Ser Thr Glu
120 130
Gln Ala Asn Thr Lys Thr Asp Ala Lys Ile Ser Asn Thr
140
Thr Ala Thr Thr Gln Asn Ser Thr Asp Ile Phe Glu Gly

Gly Gly Lys

Another subject of the present invention is monoclonal and polyclonal antibodies directed against the peptides and the polypeptide according to the invention.

Another subject of the present invention is hybridomas producing monoclonal antibodies directed against the peptides I to VII and the polypeptide according to the invention.

Another subject of the present invention is a procedure for the assay or detection of the peptides and polypeptides according to the invention in tissues and biological fluids which comprises the utilization of monoclonal or polyclonal antibodies according to the invention.

For this purpose, it is possible to utilize in particular a method of the RIA type using a peptide labelled by a radioisotope, and the competition between this peptide and the peptide to be assayed (Niswender G.D. et al; Proc. Soc. Exp. Biol. 128, 807, 1968). It is also possible to utilize a method of the ELISA type using, for example, a peptide bound to a support and the competition between this peptide and the peptide to be assayed for antibodies prepared against this peptide. The antibodies retained by the peptide bound to the support are detected by antibodies directed against the former and linked to an enzyme (method derived from Avromeas J. and Guilbert B, C.R. Acad. Sci. Paris 1971, 273, 2305).

The peptides according to the present invention may be prepared in a standard manner by peptide synthesis in liquid or solid phase by successive couplings of the different amino acid residues to be incorporated (from the N-terminal end toward the C-terminal end in liquid

phase, or from the C-terminal end to the N-terminal end in solid phase) and the N-terminal ends and the reactive side chains of which are blocked beforehand by standard groupings.

For the synthesis in solid phase it is possible to utilize in particular the technique described by Merrifield, in the article entitled "Solid phase peptide synthesis" (J. Am. Chem. Soc., 85, 2149- 2154).

In order to produce a peptide chain according to the Merrifield procedure, recourse is had to a very porous polymeric resin, to which the first C-terminal amino acid of the chain is bound. This amino acid is bound to the resin through the intermediary of its carboxyl group and its amine function is protected, for example by the t-butoxycarbonyl group.

When the first C-terminal amino acid is thus bound to the resin, the protecting group is removed from the amine function by washing the resin with an acid. In the case in which the protecting group for the amine function is the t-butoxycarbonyl group, it may be removed by treatment of the resin with the aid of trifluoroacetic acid.

The second amino acid, which provides the second residue of the desired sequence, is then coupled to the deprotected amine function of the first C-terminal amino acid bound to the chain. Preferably, the carboxyl function of this second amino acid is activated, for example by means of dicyclohexylcarbodiimide, and the amine function is protected, for example by the t-butoxycarbonyl.

In this way, the first part of the desired peptide chain is obtained which contains two amino acids and the terminal amine function of which is protected. As previously, the amine function is deprotected and it is then possible to proceed to the attachment of the third residue under similar conditions to those for the addition of the second C-terminal amino acid.

In this manner, the amino acids which will constitute the peptide chain are attached one after the other to the amine group, deprotected beforehand each time, of the portion of the peptide chain already formed and which is attached to the resin.

When the whole of the desired peptide chain is formed, the protecting groups are removed from the different amino acids constituting the peptide chain and the peptide is cleaved from the resin, for example with the aid of hydrogen fluoride.

The peptide thus obtained can be purified, for example by means of column chromatography.

The SMR1 peptide or derivatives of this peptide can also be obtained with the aid of the techniques of genetic engineering; they can also be obtained by purification from biological material by means of techniques of chromatography or precipitation similar to those used, for example, for the purification of human growth hormone starting from the hypophysis.

The monoclonal and polyclonal sera can be prepared according to a standard technique. For this purpose the tetrapeptides or the pentapeptides or multimeric derivatives of these peptides can be coupled to immunogenic agents such as KLH (Keyhole Lympet Hemocyanin), ovalbumin, bovine serumalbumin etc, by a coupling agent such as glutaraldehyde. The SMR1 protein and the derivatives of this protein (peptides derived from this protein, or hybrid proteins containing a part or all of SMR1 linked to another protein such as protein A) can also be injected directly.

The immunizations can be performed in a standard manner, for example, in the rabbit and the mouse by injecting into the animal 100 micrograms, for example, of the coupling product in the presence of Freund's adjuvant 3 to 4 times at intervals of 3 weeks. It is thus possible to obtain polyclonal sera in the rabbit.

The hybridomas and the monoclonal antibodies can be obtained by means of the standard procedures.

The isolation of the mRNA, which corresponds to the SMR1 polypeptide, and the properties of the polypeptide and the maturation products will be described in more detail below.

1) MATERIALS AND METHODS

Animals and hormonal treatments

10 weeks old male and female Wistar rats were obtained from Iffa-Credo. The androgens were removed by castration and 10 days later 35 mg of testosterone (Sterandryl retard, Roussel) were injected by the intraperitoneal route in the cases indicated. In the cases indicated, the same dose of testosterone was administered to female rats. 8 weeks old DBA/2 and "Swiss" mice were obtained from the Pasteur Institute.

Extraction of the RNA and in vitro translation

The RNA was prepared from rat and mouse tissues as described in the literature (Tronik D. et al. 1987, EMBO J. 6, 983-987). The in vitro translation of the RNAs was carried out by using the mRNA- dependent translation system as a lysate in the presence of mRNA-dependent reticulocytes (Pelham H. RB et al., 1976, Eur. J. Biochem. 67, 247-256). The products were analyzed by electrophoresis on a denaturing polyacrylamide-NaDodSO_4 gel.

Cloning and characterization of the cDNA coding for SMR1

Poly(A) RNA obtained from SMG of male Wistar rats was used as matrix for the reverse transcriptase, and the double-stranded cDNAs were obtained by means of the DNA synthesis system of Amersham, with the protocol supplied by the manufacturer. The double-stranded cDNA was then inserted into the PstI site of pUC9 by the method of the oligo-d(C) ends (Maniatis, T. et al. (1982) in Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) pp 241-242). Host bacteria (bacterial strain DH 5-1 derived from DH1 and yielding high efficiencies of transformation, see

Hanatian DNA cloning vol. 1, p. 111) were transformed and the colonies were screened by hybridization with the probes described below. In brief, the mRNA derived from the SMG of males and females was fractionated in a 5-20% sucrose gradient. The mRNA-enriched fractions of low molecular weight (which were shown by in vitro translation to contain the mRNA coding for SMR1) were precipitated with ethanol, and they were used as matrix for the reverse transcriptase in the presence of dGTP and dCTP radiolabeled with ^{32}P. About 3000 recombinants were screened on filters in duplicate samples with the radiolabeled cDNA obtained from the SMG of male and female rats. The clones selected demonstrated a strong hybridization with the male cDNA probe but a very weak hybridization with the female probe. The recombinant clones were identified by experiments of inhibition of translation of the mRNAs in a cell-free system by DNA-mRNA hybridization (Paterson et al. (1977) Proc. Natl. Acad. Sci. USA, 74, 4370-4374).

Sequencing of the cDNA coding for SMR1

With the aid of various restriction enzymes, the cDNA coding for SMR1 was cut and the fragments obtained were subcloned in the vector M13 mp9. The sequencing of the DNA was carried out by means of the method of termination with dideoxyribonucleotides (Sanger, F. et al. (1977) Proc. Natl. Acad. Sci. USA, 74, 5463-5467).

2) RESULTS

Analysis of the in vitro translation of mRNA prepared from the SMG of rats and mice.

5 μg of total RNA obtained from the SMG of male and female mice or male and female rats were translated in a cell-free system of reticulocytes in the presence of ^{35}S-methionine. The products of in vitro translation were subjected to electrophoresis on a 12.5% sodium dodecylsulfate-polyacrylamide gel and autoradiographed.

The comparison of the products of the in vitro translation of RNA prepared from the SMG of male and female rats shows the presence of several polypeptides (of apparent molecular weights of 18,000, 19,000, 35,000, 46,000) in larger amounts in the male rats than in the females. These data show the existence of a sexual dimorphism in the SMG of the rat.

Furthermore, the comparison of these products of translation with those obtained with RNAs derived from SMG of mice shows that most of the major polypeptides are different in the two species. In particular, whereas sex-linked differences could also be observed in the mouse, these differences did not relate to the same polypeptides as in the rat. Conversely, the products of translation observed in the rat to be specific for the male seem to be absent in the mouse.

Isolation and sequence of a cDNA complementary to a specific mRNA of the male rat

In order to isolate mRNAs specific for the male from the SMG of the rat, a bank of cDNA prepared from this tissue was constructed in pUC9. The recombinant clones were screened by utilizing a differential screening strategy such as that described in the Materials and Methods section. The positive clones were characterized by experiments of inhibition of translation of the mRNAs in a cell system by DNA-mRNA hybridization. One category of recombinant cDNAs suppressed the in vitro synthesis of a polypeptide having an apparent molecular weight of 19,000 daltons. This polypeptide, designated SMR1, is present in the products of in vitro translation of RNAs from male rats but is not present in those from females. The corresponding cDNA was utilized as probe in RNA hybridization experiments with transfer to a nitrocellulose membrane. For the hybridization a solution was used containing:

- 0.5 M sodium phosphate, pH 7.2
- 7% sodium dodecylsulfate (SDS)

- 1 mM EDTA
- 1% bovine serum albumin
- sonicated salmon sperm DNA: 100 mg/ml

The experiment was done at 65°C for 16 to 20 h.

4 washings of 10 minutes each were carried out at 65°C with a solution containing:

40 mM sodium phosphate, pH 7.2

- 1% SDS
- 1 mM EDTA

The cDNA is hybridized with an mRNA 700 nucleotides long, present in large amounts in the SMG of male rats. This inserted element of cDNA has been characterized in more detail.

The sequence of the cDNA coding for SMR1 and the sequence deduced for the protein are represented below

```
AAA CTG ACT CAC CAG AGA GCT TCT CAC CAG CAC ATT TCC CCG CTC AGA AGT TTC      54

                        1
                   Met Lys Ser Leu Tyr Leu Ile Phe Gly Leu Trp Ile
TCC AAG CGG CTA CCA AAG ATG AAG TCA CTG TAT TTG ATC TTT GGC CTG TGG ATC     108

                       20                                        30
Leu Leu Ala Cys Phe Gln Ser Gly Glu Gly Val Arg Gly Pro Arg Arg Gln His
CTT CTA GCA TGC TTC CAG TCA GGT GAG GGT GTC AGA GGC CCA AGA AGA CAA CAT     162

                                   40
Asn Pro Arg Arg Gln Gln Asp Pro Ser Thr Leu Pro His Tyr Leu Gly Leu Gln
AAT CCT AGA AGA CAA CAA GAT CCT TCA ACT CTT CCT CAT TAT CTT GGT CTT CAG     216

     50                                  60
Pro Asp Pro Asn Gly Gly Gln Ile Gly Val Thr Ile Thr Ile Pro Leu Asn Leu
CCT GAT CCC AAT GGT GGA CAA ATA GGA GTA ACA ATC ACT ATA CCC TTA AAT CTT     270

               70                                  80
Gln Pro Pro Arg Val Leu Val Asn Leu Pro Gly Phe Ile Thr Gly Pro Pro Leu
CAA CCA CCT CGT GTT CTT GTT AAT CTT CCC GGT TTT ATC ACT GGA CCA CCA TTG     324

                   90                                      100
Val Val Gln Gly Thr Thr Glu Tyr Gln Tyr Gln Trp Gln Leu Thr Ala Pro Asp
GTT GTA CAA GGT ACC ACT GAA TAT CAA TAT CAG TGG CAG CTA ACT GCT CCA GAC     378

                        110                                     120
Pro Thr Pro Leu Ser Asn Pro Pro Thr Gln Leu His Ser Thr Glu Gln Ala Asn
CCT ACA CCT CTA AGC AAT CCT CCT ACT CAA CTT CAT TCC ACA GAA CAA GCA AAT     432

                                130
Thr Lys Thr Asp Ala Lys Ile Ser Asn* Thr Thr Ala Thr Thr Gln Asn* Ser Thr
ACA AAA ACA GAT GCC AAA ATC TCC AAC ACT ACT GCG ACT ACC CAA AAT TCC ACT     486

    140
Asp Ile Phe Glu Gly Gly Gly Lys
GAT ATT TTT GAA GGT GGT GGC AAA TAATAAATTCCTTTTGCCAGTTACAATAGCATAAATCAA     549

AACACTGTGTAGTTTTGCGCCAAATAATCTTTAAAGCGTTCAGAAACAACCTTTACCCCCATTATAGAAAA     620

TGACAATAAAGAGCTAAGCAGCATTACACAGCAAAAAA                                    658
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The inserted cDNA has a length of 652 nucleotides, if the poly(A) fragment is excluded. The sequence comprises an open reading frame of 510 nucleotides. The only ATG which can serve as initiation codon is situated 73 nucleotides downstream from the 5' end of the clone of cDNA. The untranslated region at the 3' end (142 nucleotides long) contains the consensus signal for polyadenylation (AATAAA), 23 nucleotides upstream from the poly(A) tail (nucleotides 625-630). The translation stop codon is found in another AATAAA sequence (512- 517) which apparently is not recognized as polyadenylation signal.

The corresponding protein has a length of 146 amino acids, which corresponds to a molecular weight of about 16,000 daltons. This molecular weight is slightly lower than that determined from the electrophoretic mobility of the in vitro translation product (19,000 daltons). Such a difference between the molecular weight calculated from the analysis of the sequence and that determined from the electrophoretic mobility has already been described for other proteins of the SMG of the rat or the mouse.

SMR1 has a relatively high content of glutamine and proline residues, but does not contain a repetitive region. Hence, it does not belong to the family of polypeptides "rich in proline" or "rich in glutamine", which are essential proteins of the SMG. Furthermore, the sequence of the mRNA does not contain at its 5' end the sequence of 80 nucleotides which is characteristic of this family. However, like these proteins, SMR1 does not contain cystein or methionine residues (except in its signal peptide).

The amino-terminal part of SMR1 is strongly hydrophobic, which is characteristic of signal peptides of most of the secreted proteins. Although the amino-terminal sequence of the mature protein has not been determined directly, from the statistical analysis made according to G. Von Heijne (Nucleic Acids Res. 14,

4683-4690, 1986) (rule "-3,-1"), it may be supposed that the cleavage site of the signal peptide is located between the residues 18 and 19.

The protein SMR1 also shows certain features characteristic of the glycoproteins. The presence of two potential glycosylation sites linked to N are observed at the positions 139 and 136. The protein is relatively rich in proline (12%), threonine (12%) and glutamine (9.5%). Several glycosylation sites linked to O might thus be present in the carboxyterminal fragment of SMR1, since regions rich in proline and threonine residues are usually present in highly O-glycosylated proteins, such as the mucoproteins and the sialoglycoproteins.

An interesting characteristic is the presence of pairs of basic amino acids Arg-Arg at positions 27-28 and 33-34. Such dipeptides represent potential sites of cleavage by maturation enzymes (Lazure, C., et al (1983) Can. J. Biochem., Cell Biol. 61, 501-515 and Docherty, K. et al (1982) Ann. Rev. Physiol. 44, 625-638). They flank a tetrapeptide Gln-His-Asn-Pro. The tetrapeptide and its adjacent sequences are located in a hydrophilic environment which renders this region accessible to possible maturation enzymes.

The cleavage of Arg-Arg linkages by a maturation enzyme followed by the removal of the basic residues by carboxypeptidase E (Fricker, L.D. et al (1983) J. Biol. Chem. 258, 10950-10955) and possibly an aminopeptidase (Loh, Y.P. et al (1984) Ann. Rev. Neurosci. 7, 189-222) would produce a mixture of tetrapeptide (Gln-His-Asn-Pro) and pentapeptide (Gln-His-Asn-Pro-Arg), since "Pro-Arg" is not a good substrate for carboxypeptidase E. Other post-translational modifications could also include the formation of pyroGlu acid derivatives of these products, giving rise to a mixture of pyroGlu-His-Asn-Pro-Arg and pyroGlu-His-Asn-Pro. These structures recall those of thyroliberin (TRH).

Regulation of the accumulation of mRNA coding for SMR1 in the SMG of the rat by androgens.

In order to study the regulation of the accumulation of mRNA coding for SMR1 in the SMG of the rat by androgens, mRNA containing a poly(A) sequence was prepared from the SMG of adult males, males castrated 20 days previously, castrated males subjected to a treatment with androgens, females and females treated with androgens. 1 μg of total RNA from male rats, female rats treated with testosterone, castrated male rats, castrated male rats treated with testosterone and female rats was subjected to electrophoresis on a 1.4% agarose-formaldehyde gel, these RNAs were transferred to a Nylon membrane and were hybridized with the cDNA probe coding for SMR1. The time of exposure for autoradiography was 2 hours. The results of the RNA analysis, by transfer of these mRNAs to solid supports, by means of an SMR1 probe labeled with ^{32}P are shown in the figure (part A). A considerable difference in the accumulation of mRNA coding for SMR1 is observed in the SMG of male rats and those of females.

Furthermore, various amounts (as indicated in the figure) of RNA from the SMG of male rats and female rats were subjected to electrophoresis on a 2% agarose-formaldehyde gel, they were transferred to filters and hybridized with the cDNA probe coding for SMR1. The film was exposed for 30 hours. As is apparent in part B, the mRNA coding for SMR1 accumulates in very large quantities in the SMG of male Wistar rats, since an amount as low as 1.5 ng of total RNA was sufficient to give a hybridization signal. Conversely, the level of accumulation of mRNA coding for SMR1 in the SMG of female Wistar rats was about 1,000 to 3,000 times lower than that in males.

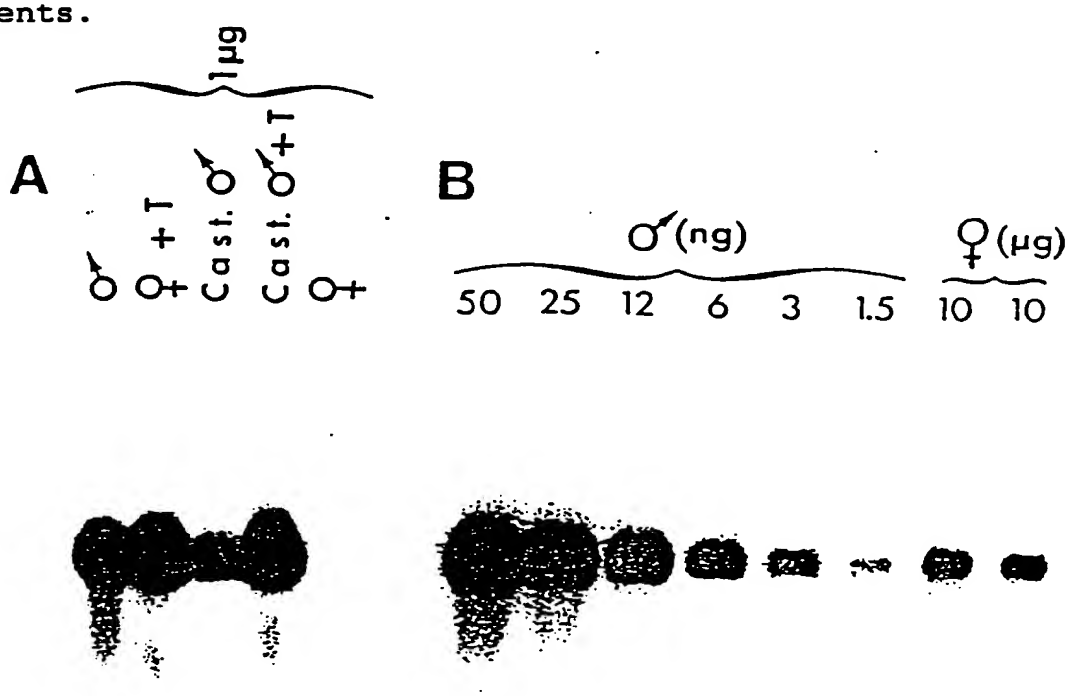
In the castrated males, the quantity of mRNA coding for SMR1 was reduced 10 to 20 fold. The administration of testosterone to these males restored the amount of mRNA coding for SMR1 to the same value as that observed in the non-castrated males. Furthermore,

the administration of testosterone to adult female rats caused the accumulation of mRNA coding for SMR1 in amounts similar to that observed in the males.

A remarkable property of the mRNA coding for SMR1 is its high degree of accumulation in the SMG of the rat in response to a treatment with androgens. This strongly suggests that SMR1 is synthesized in the GCT cells of the SMG (like the EGF, NGF and renin as well as other proteins under the control of androgens in the SMG of the mouse). Furthermore, the difference in the level of accumulation of mRNA coding for SMR1 in the male and in the female is very great (greater than three orders of magnitude), in comparison with that usually observed for other genes controlled by androgens in the target organs (kidney, liver, SMG).

These results, and in particular the high degree of induction of the SMR1 gene by androgens, suggest that SMR1 may fulfil an important function specific for the male in the rat. SMR1 might be the precursor of a molecule (the tetra- or pentapeptides or the C-terminal part of SMR1) controlling behavioral characteristics in the male rat.

The products described in the present invention can be used for therapeutic purposes or as laboratory reagents.



CLAIMS

1. Peptide of formula:

X-His-Asn-Pro-Y I

in which:

X represents a Gln or pyro-Glu residue,

Y represents an OH group or a residue of a basic amino acid.

2. Peptide according to Claim 1, in which Y is a Lys or Arg residue.

3. Peptide of formula:

Gln-His-Asn-Pro II

4. Peptide of formula:

pyro-Glu-His-Asn-Pro III

5. Peptide of formula:

Gln-His-Asn-Pro-Arg IV

6. Peptide of formula:

pyro-Glu-His-Asn-Pro-Arg V

7. Peptide of formula:

Gln-His-Asn-Pro-Lys VI

8. Peptide of formula:

pyro-Glu-His-Asn-Pro-Lys VII

9. Polypeptide of formula:

1
Met Lys Ser Leu Tyr Leu Ile Phe Gly Leu Trp Ile Leu
20
Leu Ala Cys Phe Gln Ser Gly Glu Gly Val Arg Gly Pro
30
Arg Arg Gln His Asn Pro Arg Arg Gln Gln Asp Pro Ser
40 50
Thr Leu Pro His Tyr Leu Gly Leu Gln Pro Asp Pro Asn
60
Gly Gly Gln Ile Gly Val Thr Ile Thr Ile Pro Leu Asn
70
Leu Gln Pro Pro Arg Val Leu Val Asn Leu Pro Gly Phe
80 90
Ile Thr Gly Pro Pro Leu Val Val Gln Gly Thr Thr Glu
100

Tyr Gln Tyr Gln Trp Gln Leu Thr Ala Pro Asp Pro Thr
110
Pro Leu Ser Asn Pro Pro Thr Gln Leu His Ser Thr Glu
120 130
Gln Ala Asn Thr Lys Thr Asp Ala Lys Ile Ser Asn Thr
140
Thr Ala Thr Thr Gln Asn Ser Thr Asp Ile Phe Glu Gly

Gly Gly Lys

and derivatives of this polypeptide.

10. Monoclonal and polyclonal antibodies directed against a peptide or polypeptide according to any one of the Claims 1 to 9.

11. Hybridomas characterized in that they produce monoclonal antibodies according to Claim 10.

12. Assay or detection procedure for a peptide according to any one of the Claims 1 to 8, comprising the utilization of monoclonal antibodies directed against these peptides.

13. Assay procedure for a polypeptide according to Claim 9, comprising the utilization of monoclonal antibodies directed against this polypeptide.

14. Therapeutic composition containing a peptide or a polypeptide according to any one of the Claims 1 to 9.